UNIVERSITY OF THESSALY

SCHOOL OF MEDICINE

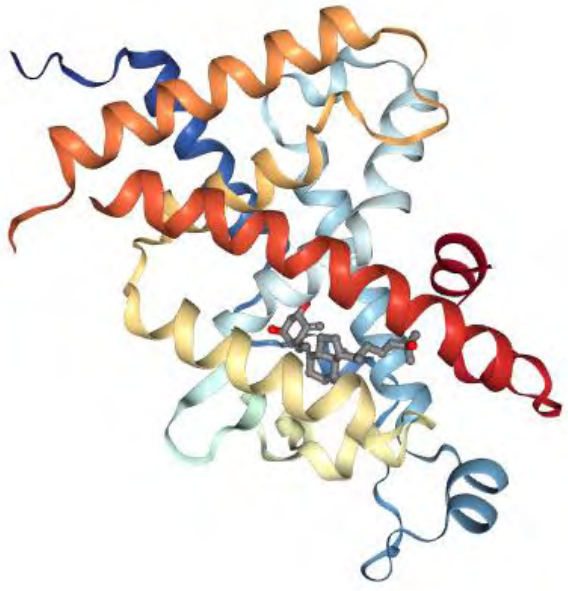
LABORATORY OF BIOINFORMATICS

MASTER PROGRAM IN

"RESEARCH METHODOLOGY IN BIOMEDICINE, BIOSTATISTICS AND CLINICAL BIOMATHEMATICS"

MASTER THESIS

# "ASSESSMENT OF GENE ASSOCIATION STUDIES FOR VITAMIN D RECEPTOR POLYMORPHISMS & DIABETES TYPE 2"



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ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ

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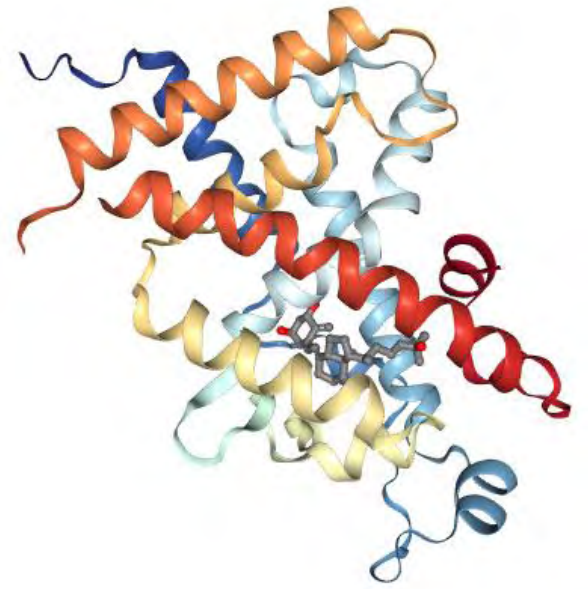
ΕΡΓΑΣΤΗΡΙΟ ΒΙΟΜΑΘΗΜΑΤΙΚΩΝ

ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ

«ΜΕΘΟΔΟΛΟΓΙΑ ΒΙΟΪΑΤΡΙΚΗΣ ΕΡΕΥΝΑΣ, ΒΙΟΣΤΑΤΙΣΤΙΚΗΣ ΚΑΙ ΚΛΙΝΙΚΗΣ ΒΙΟΠΛΗΡΟΦΟΡΙΚΗΣ»

ΔΙΠΛΩΜΑΤΙΚΗ ΕΡΓΑΣΙΑ

# «ΑΞΙΟΛΟΓΗΣΗ ΜΕΛΕΤΩΝ ΓΕΝΕΤΙΚΗΣ ΣΥΣΧΕΤΙΣΗΣ ΑΝΑΜΕΣΑ ΣΤΟΥΣ ΓΟΝΙΔΙΑΚΟΥΣ ΠΟΛΥΜΟΡΦΙΣΜΟΥΣ ΤΟΥ ΥΠΟΔΟΧΕΑ ΤΗΣ ΒΙΤΑΜΙΝΗΣ D & ΤΟΥ ΔΙΑΒΗΤΗ ΤΥΠΟΥ 2»



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# Abstract

Introduction

Type 2 diabetes is the most common form of diabetes affecting a large population worldwide. Vitamin D receptor (VDR) is a main transcription factor that has been linked to type 2 diabetes.

Objective

The aim of this study is to assess the Genetic Association Studies (GAS) referred to the Vitamin D Receptor (VDR) polymorphisms TaqI, BsmI, FokI and ApaI and type 2 diabetes mellitus (T2DM).

Methods

After an extended search of genetic association studies, each study was assessed – according to the standards set by literature, described in detail – and then a meta-analysis was performed for each polymorphism.

Results

The meta-analysis involved 13 studies, for TaqI polymorphism 8 studies were included (1134 cases + 978 controls). Overall, the results indicate that heterozygotes (**TC**) are protected of type 2 diabetes – there is a smaller chance for the heterozygotes to be affected by the disease than for the homozygotes (**TT+CC**) – as indicated by the OR that was **significant** [FE OR=0.776/ 95% CI = (0.625, 0.964) / RE OR=0.771 / 95% CI = (0.600, 0.991)]. There was **no heterogeneity** ($I^2$ =22.55 %), meaning variations are due to chance.

For BsmI polymorphism, 7 studies were included in the meta-analysis (1067 cases + 1466 controls). In **subgroup analysis** (4 case-control studies/315 cases & 356 controls) for Caucasians – with dark skin [including Indians and Moroccans but not Hui Chinese (descendants of Arabic & Persian merchants)] the results indicate that carriers of the A allele (AA+AG) have a higher risk of acquiring type 2 diabetes – as indicated by the OR that was **significant** [FE OR=1.4824 / 95% CI = (1.0309, 2.1317) /RE OR=1.494 / 95% CI = (1.017, 2.196)]. There was **no heterogeneity** ($I^2$ = 8.07 %). When Hui Chinese were included in the subgroup of Caucasians – with dark skin, the results were not significant and there was medium heterogeneity ($I^2$ = 35.7 %). **But, after repeating the analysis – without the study that included 30 (out of 40) related patients – no significant associations were found and there was no heterogeneity since $I^2$<25%.**

For FokI polymorphism, 7 studies were included in the meta-analysis (1960 cases + 1965 controls). In **subgroup analysis** (2 case-control studies/ 298 cases & 332 controls) for the mixed population of Chile [35% Caucasians, 60% Castizos/ Mestizos = 60% Caucasians and 40% native Americans, 5% native Americans] the results indicate that: a) heterozygotes CT have a higher risk of acquiring type 2 diabetes compared to the homozygotes (CC+TT) – as indicated by the OR that was **significant** [FE OR=1.775, 95% CI = (1.2916, 2.4393) /RE OR=1.747, 95% CI = (1.612, 1.893)]. There was **no heterogeneity** ($I^2$ = 0%), b) heterozygotes CT have a higher risk of acquiring type 2 diabetes compared to the homozygotes CC – as indicated by the OR (CC vs CT) that was **significant** [FE OR=0.4563, 95% CI = (0.3007, 0.6842)]. There was **medium heterogeneity** ($I^2$ = 34.74%).

For ApaI 3 studies were included in the meta-analysis (473 cases + 429 controls) but no statistically significant association was detected.

Conclusion

It would be useful to further investigate the possible associations of the 4 VDR polymorphisms with type 2 diabetes, especially TaqI polymorphism and FokI in mixed populations like the one in Chile (possibly native American ancestry may explain the differences), that showed a significant association.

## Περίληψη

Εισαγωγή

Ο διαβήτης τύπου 2 είναι η πιο συχνή μορφή διαβήτη που επηρεάζει μεγάλο μέρος του πληθυσμού παγκοσμίως. Ο υποδοχέας της βιταμίνης D (VDR) είναι ένας κύριος μεταγραφικός παράγοντας που έχει συνδεθεί με τον διαβήτη τύπου 2.

Στόχοι

Στόχος της μελέτης είναι η αξιολόγηση των Μελετών Γενετικής Συσχέτισης που αναφέρεται στους πολυμορφισμούς της VDR TaqI, BsmI, FokI και ApaI και τον διαβήτη τύπου 2.

Μέθοδοι

Μετά από εκτεταμένη αναζήτηση μελετών γενετικής συσχέτισης, κάθε μελέτη αξιολογήθηκε – σύμφωνα με τις προδιαγραφές που ορίζονται από τη βιβλιογραφία και αναλύονται λεπτομερώς – και κατόπιν πραγματοποιήθηκε μετα-ανάλυση για κάθε πολυμορφισμό.

Αποτελέσματα

Για τον πολυμορφισμό TaqI, 8 μελέτες περιλήφθηκαν στην μετα-ανάλυση (1134 ασθενείς+978 υγιείς). Συνολικά, τα αποτελέσματα υποδεικνύουν ότι οι ετερόζυγοι (TC) είναι προστατευμένοι από τον διαβήτη τύπου 2, καθώς υπάρχει μικρότερη πιθανότητα οι ετερόζυγοι να εμφανίσουν την ασθένεια συγκριτικά με τους ομόζυγους (TT+CC) σύμφωνα με το OR που ήταν **στατιστικά σημαντικό** [FE OR=0.776/ 95% CI = (0.625, 0.964) / RE OR=0.771 / 95% CI = (0.600, 0.991)]. **Δεν παρατηρείται ετερογένεια**, δηλαδή οι διακυμάνσεις οφείλονται στην τύχη (I=22.5%).

Για τον πολυμορφισμό BsmI, 7 μελέτες περιλήφθηκαν στην μετα-ανάλυση (1067 ασθενείς + 1466 υγιείς). Στην ανάλυση υποομάδων (4 μελέτες/315 ασθενείς & 356 υγιείς) σε Καυκάσιους – με σκούρο δέρμα [Ινδοί και Μαροκινοί αλλά όχι οι Κινέζοι Hui (που είναι απόγονοι Αράβων & Περσών εμπόρων)] τα αποτελέσματα υποδεικνύουν ότι οι φορείς του αλληλομόρφου A (AA+AG) έχουν μεγαλύτερο κίνδυνο να εμφανίσουν διαβήτη τύπου 2 σύμφωνα με το OR που ήταν **στατιστικά σημαντικό** [FE OR=1.4824 / 95% CI = (1.0309, 2.1317) /RE OR=1.494 / 95% CI = (1.017, 2.196)]. **Δεν παρατηρείται ετερογένεια** ($I^2$ = 8.07 %). Όταν περιλήφθηκαν οι Hui στους Καυκάσιους τα αποτελέσματα δεν ήταν στατιστικά σημαντικά. **Όμως, αν αφαιρέσουμε τη μελέτη – όπου συμπεριελήφθησαν 30 (από τους 40) ασθενείς με συγγενικές σχέσεις – δεν βρίσκουμε στατιστικά σημαντικά αποτελέσματα ενώ δεν παρατηρείται ετερογένεια ($I^2$ <25%).**

Για τον πολυμορφισμό FokI, 7 μελέτες περιλήφθηκαν στην μετα-ανάλυση (1960 ασθενείς + 1965 υγιείς). Στην **ανάλυση υποομάδων** (2 μελέτες/ 298 ασθενείς & 332 υγιείς) σε μικτό πληθυσμό[35% Καυκάσιοι, 60% Castizos/ Mestizos = 60% Καυκάσιοι and 40% γηγενείς Αμερικανοί, 5% γηγενείς Αμερικανοί], τα αποτελέσματα υποδεικνύουν ότι: α) οι ετερόζυγοι CT έχουν μεγαλύτερο κίνδυνο να εμφανίσουν διαβήτη τύπου 2 συγκριτικά με τους ομόζυγους (CC+TT) σύμφωνα με το OR που ήταν **στατιστικά σημαντικό** [FE OR=1.775, 95% CI = (1.2916, 2.4393) /RE OR=1.747, 95% CI = (1.612, 1.893)]. Δεν παρατηρείται ετερογένεια ($I^2$ = 0%), b) οι ετερόζυγοι CT έχουν μεγαλύτερο κίνδυνο να εμφανίσουν διαβήτη τύπου 2 συγκριτικά με τους ομόζυγους CC σύμφωνα με το OR που είναι **στατιστικά σημαντικό** [FE OR=0.4563, 95% CI = (0.3007, 0.6842)]. Παρατηρείται **μέτρια ετερογένεια** ($I^2$ = 34.74%).

Για τον πολυμορφισμό ApaI 3 μελέτες περιλήφθηκαν στην μετα-ανάλυση (473 ασθενείς + 429 υγιείς) όμως δεν παρατηρήθηκε στατιστικά σημαντική σχέση.

Συμπεράσματα

Θα ήταν χρήσιμη η περεταίρω έρευνα της πιθανής συσχέτισης των 4 πολυμορφισμών με τον διαβήτη τύπου 2, ειδικά για τον πολυμορφισμό TaqI και για τον πολυμορφισμό FokI σε μικτούς πληθυσμούς όπως αυτός της Χιλής (πιθανή εξήγηση για τις διαφορές που παρατηρήθηκαν είναι η γηγενής Αμερικανική καταγωγή), καθώς δείχθηκε στατιστικά σημαντική συσχέτιση.

INTRODUCTION

For complex traits, like diabetes, association studies are more powerful than linkage because the causal risk factor is measured [1].

Diabetes mellitus is a heterogeneous metabolic disorder characterized by the presence of hyperglycemia due to impairment of insulin secretion, defective insulin action or both. The chronic hyperglycemia of diabetes is associated relatively specific long-term microvascular complications effecting the eyes, kidneys and nerves as well as increased risk of cardiovascular disease. **Type 2 diabetes** may range from predominant insulin resistance with relative insulin deficiency to a predominant secretory defect with insulin resistance. Ketosis is not as common [2].

Type 2 diabetes is associated with serious morbidity and increased mortality. Type 2 diabetes is the most common of diabetes accounting for 85-90% of all cases. Worldwide the total number of people with diabetes is expected to rise from 171 million in 2000 to 366 million by 2030 [3].

Vitamin D may play a role in modifying risk of diabetes since there has been increasing evidence from animal and human studies [4]. Vitamin D binds to the cytosolic/nuclear Vitamin D Receptor – VDR, which is a member of the steroid/thyroid hormone receptor family that functions as a transcriptional activator of many genes. VDR is expressed in tissues like muscle and pancreatic β cells that are involved in the regulation of glucose metabolism ([5], [6], [7], [8]). Additionally, the existence of a putative membrane VDR (mVDR) has been postulated [9] and it has been shown that pancreatic β cells express both specific cytosolic/nuclear VDR and the putative membrane VDR (mVDR) [10]. As stated by A.G. Pittas et al. in 2007 ([4]), vitamin D is thought to have both direct (by the activation of the vitamin D receptor) and indirect (by the regulation of calcium homeostasis) effects on various mechanisms related to the pathophysiology of type 2 diabetes, including impaired pancreatic -b cell function and insulin resistance.

As stated by Palomer X et al. in 2008 ([11]), vitamin D modulates insulin secretion it is feasible that genetic variants of the VDR gene may contribute to the development of type 2 diabetes mellitus and since patients with type 2 diabetes exhibit subtle alterations in glucose metabolism long before the onset of the disease, genetic factors contributing to its pathogenesis or development could be detected early in the disease process.

Polymorphisms are variations in the genetic code that are present in more than 1% of the population. Four polymorphisms of the VDR gene, that are described in detail, are Fok I, Bsm I, Apa I, Taq I:

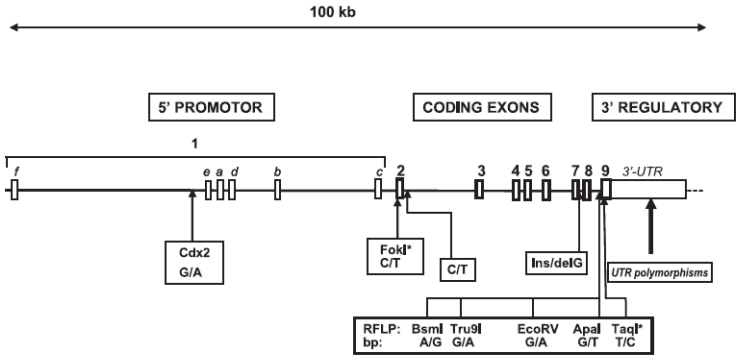


Image by Uitterlinden et al. in 2004 ([12])

Type 2 diabetes is initiated by insulin resistance and β cell dysfunction is characterized by defective insulin secretion, endoplasmic reticulum (ER) stress, eventual β cell loss and disease progression ([13], [14], [15], [16]).

Although the molecular underpinnings of obesity-induced β cell dysfunction is poorly understood, increasing evidence links inflammation and specifically the innate immune response of pancreatic islets to metabolic stress, to type 2 diabetes progression ([17], [18], [19]).

Z. Wei et al. in 2018 ([20]) identified VDR as a key modulator of inflammation and β-cell survival, so an unusual therapeutic strategy was uncovered in which the inflammation could be suppressed via sustained VD receptor activation in β-cells.

METHODS

LITERATURE SEARCH

The following searched criterion was used: “VDR” or “BsmI” or “TaqI” or “ApaI” or “FokI” or “BsmI” or “Taq1” or “Apa1” or “Fok1” and “type 2 diabetes” or “T2D” or “type 2 diabetes mellitus” or “T2DM”. The genetic association studies published from 2008 to 2018, in English and in full text form, were included.

QUALITY ASSESSMENT CRITERIA

To assess a genetic association study, it is important to define the correct steps in designing and conducting such a study ([21]). Our goal is to examine the association of VDR polymorphisms and diabetes type 2. We should take under consideration, to evaluate each genetic association study ([22]) the following matters:

1. The gene of interest should be chosen based on a certain biological mechanism.
2. The sequence of normal **variants** should be determined as well as the **frequency** in certain populations of interest.
3. The **effect of the polymorphism** (frequency>1%) on the function of the gene should be mentioned and cited.
4. **Haplotype analysis**. The genotyping of a specific combination of polymorphisms that represent all common **haplotypes** (frequency>5% in the population under study) helps to ensure that the entire gene is represented in the analysis. Polymorphisms found to be associated with a disease may be important themselves or may be in linkage disequilibrium with the important variant or combination of variants.
5. Defining the **phenotype**. It is important to define the phenotype based on physiologic and clinical criteria. This enables replication of the genetic association study.
6. Definition of the **type of study**: case-control, cohort study, family study.
7. **Genotyping error**. First and foremost, we need valid genotyping methods like TaqMan, Sequenom (mass spectrometry), rapid throughput sequencing. To avoid genotyping error, two independent genotypers should be employed & they should be blinded to the case-control status of the subject. Additionally, a repetition of the genotyping in a randomly chosen subgroup of subjects using the same or a different technique to ensure genotyping accuracy.
8. **Matching cases & controls. Choosing the cases and the controls** according to specific criteria & more details like age, gender etc. could provide homogeneity to the groups. In case of known gene-environment interaction the ideal control group would be exposed to the relevant environmental influences but free from the case condition.

   Confounders

9. **Population stratification**. Cases and controls should be matched for ethnicity because otherwise allele frequencies may vary by **ethnicity** leading to artificial associations. But in diverse areas of the world, like United States, this problem could be addressed by:
   - genotyping a large set of unlinked markers in all cases and controls,
   - by using family-based association studies and the transmission disequilibrium test, in which two parents and the case patient are genotyped to determine the rate of transmission of the candidate gene polymorphism alleles to the affected offspring. In the transmission disequilibrium test a heterozygous parent is expected to transmit either allele 50% of the time. In a cohort of affected children, if the transmission of an allele is statistically different from the expected 50%, then this polymorphism is significantly associated with disease susceptibility.
   - genotyping of ancestry informative markers (AIMs). Also, the large number of SNPs identified in GWAS studies provides another mechanism for performing this analysis.
10. Confounding factors as age, gender and other uncontrolled factors should be analyzed as well by performing **multivariate analysis** for their effect on the results of the study.
11. **Hardy-Weinberg Equilibrium** is a formula that describes the genotype frequencies in a population and can be used to track their changes from one generation to the next. If the control group is not in Hardy-Weinberg Equilibrium we conclude that there has been genotyping errors, inbreeding, genetic drift, new mutations or population substructure.

12. **Population size determination**. The number of cases required to detect positive associations depends on the frequency of the less common allele, so a **power** calculation should be performed.

13. When

    - genotyping cases and controls studied previously for other polymorphisms,
    - more than one phenotype is analyzed in the study,
    - more than two genes or more than two alleles are under study,

    **multiple comparisons** are needed.

14. In the case of multiple comparisons, the potential for type I error increases. Addressing the problem by **correction** (Bonferroni) or via **repeating** a positive association in a separate set of cases and controls to confirm the finding so that the likelihood of a spurious association be reduced.

15. **Replication**. Replication of a genetic association is considered when the association is repeated by different investigators in different populations, for example the same SNP with the exact same phenotype in two or more populations ([23]). Criteria for replication, as stated by NCI-NHGRI Working Group ([24]) on replication in association studies, are:

    - **sufficient sample size**, to convincingly distinguish the proposed effect from no effect
    - **independent data sets**, to avoid the tendency to split one well-powered study into two less conclusive ones
    - the **same or similar phenotype** should be analyzed
    - a **similar population** should be studied and notable differences between the populations studied in the initial and attempted replication studies should be described
    - **similar magnitude of effect and significance** should be demonstrated, in the same direction, with the same SNP or a SNP in perfect or very high linkage disequilibrium with the prior SNP ($r^2$ close to 1)
    - statistical significance should be first obtained using the **genetic model** reported in the initial study
    - when possible, **a joint or combined analysis** should lead **to a smaller P- value** than that seen in the initial report
    - a **strong rationale** should be provided for selecting SNPs to be replicated from the initial study, including linkage disequilibrium structure, putative functional data or published literature
    - the level of **detail for study design and analysis plan** should be the same as reported in the initial study

In this case specifically the **phenotype** is **type 2 diabetes**.

The *clinical features* of type 2 diabetes – distinguishing it from type 1 diabetes & monogenic diabetes – are: 1) **the age of onset** is usually >25 (but incidence increasing in adolescents, paralleling increasing rate of obesity in children and adolescents), 2) **weight**, >90% at least overweight (overweight when BMI≥25kg/$m^2$ or BMI≥23kg/$m^2$ for Asians ), 3) **islet autoantibodies**, absent, 4) **C-peptide**, normal/high, 5) **insulin production**, present, 6) **family history of diabetes**, frequent (75%-90%), 7) **diabetic ketoacidosis**, rare, 8) **first line treatment**, noninsulin antihyperglycemic agents (gradual dependence on insulin may occur).

The *diagnostic criteria* for type 2 diabetes according to World Health Organization (WHO) are 1) **fasting glucose** (*fasting* is defined as no caloric intake for at least 8 h) ≥ 7mmol/L (126mg/dL) [normal: ≤ 6mmol/L but truly normal is probably<5,6mmol/L], 2) **2hr glucose in OGTT** ≥ 11,1mmol/L (200mg/dL) [normal: ≤ 7,7mmol/L], 3) **HbA1c** ≥ 48mmol/mol (6,5%) [normal: < 42mmol/mol], 4) In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis a **random plasma glucose** ≥ 11,1mmol/L (200mg/dL). ([2], [25])

STUDY ELIGIBILITY CRITERIA (FOR THE META-ANALYSIS)

In the meta-analysis were included only: 1) case-control studies, 2) studies with distribution of genotypes, in cases and control groups, 2) studies in which the diagnosis of type 2 diabetes was conducted using valid published criteria and the control group is consisted of healthy or non-diabetic individuals, 3) studies in which valid genotyping methods were used, 4) studies in which controls are in Hardy-Weinberg Equilibrium.

STATISTICAL METHODS

The genetic models we examine in this meta-analysis are: 1) the **allele contrast** model, where the numbers of the risk allele T is compared with that of allele C (**T vs C**), 2) the **recessive** model, in which the TT genotype is compared with the combined TC+CC genotype (**TT vs TC+CC**), 3) the **dominant** model, in which the combined TT+TC genotype is compared with the CC genotype (**TT+TC vs CC**), 4) the **additive** model, in which TT genotype is compared with CC genotype (**TT vs CC**), 5) the **co-dominant** model, in which the combined TT+CC is compared with TC (**TC vs TT+CC**). **Over-dominance** or **Under-dominance** was established via the **h-index** (Zintzaras E. et al. 2011 [27]). If h<-1→ under-dominance, h>+1→ overdominance.

The existence of heterogeneity (determine whether differences between the studies exist or whether variations are due to chance) was tested using 1) the **Cochran's Q test** → P value<0,10 indicates the presence of heterogeneity, 2) **$I^2$ value**, which quantifies the effect of heterogeneity without depending on the number of studies→ 0-25% - low, 26-50% - moderate, 75-100% - high. Heterogeneity is present when P value(Q)<0,1 & $I^2$>25%.

**Fixed Effects** model assumes that 1) the genetic factors have similar effects on disease susceptibility in all the studies 2) the observed variations between studies are caused by chance alone.

**Random Effects** model assumes that different studies exhibit substantial diversity and assesses intra-study sample errors and inter-study variances.

When heterogeneity exists (**Q test** → P value<0,10), **Random Effects** model is used. When **Q test** → P value>0,10 **Fixed Effects** model & **Random Effects** model would offer similar results.

**Publication Bias** (the studies that show no significant result remain unpublished) may result in the overestimation of the actual effect. The existence of the publication bias was checked with **Egger's linear regression test** (P value ≤ 0.05 → significant publication bias).

This meta-analysis was conducted with the free software MetaGenyo ([28]).

RESULTS

QUALITY ASSESSMENT

| | | | | | | |
|---|---|---|---|---|---|---|
| **Criteria** | H.K. Bid 2009 (India) FokI, BsmI, TaqI [34] | P.N. Mukhopad-hyaya 2010 (India) BsmI [35] | F. Dilmec 2010 (Turks) ApaI,TaqI [36] | N.M. Al-Daghri 2012 (Saudi) FokI, BsmI,TaqI, ApaI [37] | J.R. Xu 2014 (China) BsmI,TaqI [38] | A.Errouagui 2014 (Maroc) FokI,BsmI, ApaI [39] |
| **Type of study** | Case-control | Case-control | Case-control | Case-control | Case-control | Case-control |
| **Phenotype definition** | + | + | + | + Not analyzed | + | + |
| **Matching cases & controls** | Age & sex matched, shared geography | A sub-popuation, but 30 out of 40 patients were family material (**related**, they sould be unrelated) | Age, sex & body mass index (BMI) matched | Matched for ethnicity | Matched for ethnicity | Sex matched, similar ethnicity |
| **Relatedness** | Not mentioned | + | Not mentioned | Not mentioned | Not mentioned | - |
| **Population** | Probably. The | Probably. The | Probably. The | Probably. The | Probably. The | Probably. The |

| | | | | | | |
|---|---|---|---|---|---|---|
| **stratification** | genotyping of ancestry informative markers (AIMs) not done | genotyping of ancestry informative markers (AIMs) not done | genotyping of ancestry informative markers (AIMs) not done | genotyping of ancestry informative markers (AIMs) not done | genotyping of ancestry informative markers (AIMs) not done | genotyping of ancestry informative markers (AIMs) not done |
| **multivariate analysis** | - | - | - | - | - | - |
| **Frequency of variants or genotype distribution** | + | + | + | + | + | + |
| **Haplotype analysis** | - | - | - | + | + | + |
| **Valid genotyping methods (PCR & restriction enzyme, TaqMan, Sequenom, Rapid throughput Sequencing)** | + | + | + | + | + | + |
| **2 independent genotypers** | - (Not independent Members of the same lab) | - | - | - | - | - |
| **Genotypers blinded to the case-control status** | - (not stated) | - | - | - | + | - |
| **Re-sequencing in a target sub-population** | + | - | - | - | - | - |
| **Hardy Weinberg Equilibrium** | + | + | + | + | + | + |
| **Population size determination according to power** | - | - | - | - | - | - |
| **Multiple tests** | - | - | - | + | + | + |
| **Correction (Multiple** | - | - | - | + | + | + |

| | | | | | | |
|---|---|---|---|---|---|---|
| **tests)** | | | | | | |
| **Replication** | - No sample size determination according to power | - No sample size determination according to power | - No sample size determination according to power | - No sample size determination according to power | - No sample size determination according to power | - No sample size determination according to power |

| | | | | | |
|---|---|---|---|---|---|
| **Criteria** | B. Angel 2015 (Chile) FokI, [40] | E.A. Rivera-Leon 2015 (Mexico) ApaI,TaqI [41] | I.Mahjoubii 2016 (Tunisia) FokI, [42] | F. YU 2016 (China) FokI,BsmI [43] | A.Darraji 2017 (Iraq) FokI,BsmI,ApaI,TaqI[44] |
| **Type of study** | Case-control | Case-control | Case-control | Case-control | Case-control |
| **Phenotype definition** | + | + Not analyzed | + | + | + Fasting blood sugar>140mg/dl(not 126mg/dl) |
| **Matching cases & controls** | Gender comparable | - | Matched for ethnicity | Age & sex matched, HAN sub-population | Age & sex matched |
| **Relatedness** | Not mentioned | Not mentioned | - | Not mentioned | Not mentioned |
| **Population stratification** | Probably. The genotyping of ancestry informative markers (AIMs) not done | Probably. The genotyping of ancestry informative markers (AIMs) not done | Probably. The genotyping of ancestry informative markers (AIMs) not done | Probably. The genotyping of ancestry informative markers (AIMs) not done | Probably. The genotyping of ancestry informative markers (AIMs) not done |
| **multivariate analysis** | - | - | - | - | - |
| **Frequency of variants or genotype distribution** | + | + | + | + | + |
| **Haplotype analysis** | - | + Not analyzed | - | + | - |
| **Valid genotyping methods (PCR &** | + | + | + | + | + |

| | | | | | |
|---|---|---|---|---|---|
| **restriction enzyme, TaqMan, Sequenom, Rapid throughput Sequencing)** | | | | | |
| **2 independent genotypers** | - | - | + | Automatic Genotyping | - |
| **Genotypers blinded to the case-control status** | - (not stated) | - | - | Automatic Genotyping | - |
| **Re-sequencing in a target sub-population** | - | - | + | After quality control 1,6% of samples excluded | - |
| **Hardy Weinberg Equilibrium** | + | + | + | + | + |
| **Population size determination according to power** | - | - | - | - | - |
| **Multiple tests** | - | - | - | - | - |
| **Correction (Multiple tests)** | - | - | - | - | - |
| **Replication** | - No sample size determination according to power | - No sample size determination according to power | - No sample size determination according to power | - No sample size determination according to power | - No sample size determination according to power |

| **Criteria** | Z. Xia 2017 (China) ApaI,TaqI, FokI, BsmI [45] | D. Sarma 2018 (India) FokI, BsmI TaqI [46] | B. Angel 2018 (Chille) FokI, BsmI [47] |
|---|---|---|---|
| **Type of study** | Case-control | Case-control | Case-control |
| **Phenotype definition** | + | + | + |
| **Matching cases & controls** | Age & sex matched, HAN nationality | Age & sex matched, HAN nationality | - |

| | | | |
|---|---|---|---|
| **Population stratification** | Probably. The genotyping of ancestry informative markers (AIMs) not done | Probably. The genotyping of ancestry informative markers (AIMs) not done | Probably. The genotyping of ancestry informative markers (AIMs) not done |
| **multivariate analysis** | - | - | - |
| **Frequency of variants or genotype distribution** | + | + | + |
| **Haplotype analysis** | - | - | - |
| **Valid genotyping methods (PCR & restriction enzyme, TaqMan, Sequenom, Rapid throughput Sequencing)** | + | + | + |
| **2 independent genotypers** | - | - | - |
| **Genotypers blinded to the case-control status** | - | - | - |
| **Re-sequencing in a target sub-population** | - | - | - |
| **Hardy Weinberg Equilibrium** | + | - | + |
| **Population size determination according to power** | - | - | - |
| **Multiple tests** | - | - | - |
| **Correction (Multiple tests)** | - | - | - |
| **Replication** | - No sample size determination according to power | - No sample size determination according to power | - No sample size determination according to power |

META-ANALYSIS

To overcome the problem of small sample sizes and the inadequate statistical strength and precision, for each polymorphism a meta-analysis was performed to identify a genuine association. In this meta-analysis, only good quality studies (in HWE) were included.

Meta-analysis for TaqI polymorphism

| Studies | Distribution of **TaqI (rs 731236)** VDR genotype | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| First author, ethnicity, year | Cases | | | Controls | | | HWE P-value | HWE adjusted P-value |
| | TT (TT) | TC (Tt) | CC (tt) | TT (TT) | TC (Tt) | CC (tt) | | |
| H. K. Bid, India (Caucasians), 2009 | 36 | 49 | 15 | 67 | 65 | 28 | 0.085 | 0.2493 |
| F. Dilmec, Turkey (Caucasians), 2010 | 33 | 25 | 14 | 69 | 81 | 19 | 0.5112 | 0.5842 |
| R. J. Xu, China – Hui (descendants of Arabic & Persian merchants, over 12 million people in China), 2014 | 3 | 17 | 134 | 0 | 16 | 99 | 0.4227 | 0.5842 |
| R. J. Xu, China – Han (East Asians), 2014 | 0 | 19 | 182 | 1 | 25 | 188 | 0.8638 | 0.8638 |
| Al-Darraji SZ, Iraq (Caucasians), 2017 | 78 | 95 | 27 | 15 | 44 | 16 | 0.1329 | 0.2658 |
| E.-A. Rivera-Leon, Mexico (Mix), 2015 | 38 | 62 | 25 | 34 | 72 | 19 | 0.0591 | 0.2493 |
| D. Sarma, India (Caucasians), 2018 | 22 | 10 | 8 | 14 | 4 | 2 | 0.0935 | 0.2493 |
| Z. Xia, China – Han (East Asians), 2017 | 224 | 18 | 0 | 86 | 14 | 0 | 0.4516 | 0.5842 |
| Total | 434 | 295 | 405 | 286 | 321 | 371 | | |
| Total number of cases & controls | 1134 | | | 978 | | | | |

Table 1. Genotype distribution and Hardy-Weinberg Equilibrium for TaqI polymorphism

All the studies are in HWE (p≥0.05) as seen above (table 1). The results of the meta-analysis under each ***genetic model***, are: 1) allele contrast (T vs C)→ RE OR=1.0550 / 95% CI = (0.8233, 1.3520) / P value(Q) = 0.0497 / $I^2$ = 50.29%, 2) recessive model (TT vs TC+CC)→ RE OR=1.2667 / 95% CI = (0.8555, 1.8755) / P value(Q) = 0.0598 / $I^2$ = 48.34%, 3) dominant model (TT+TC vs CC)→ FE OR=0.8923 / 95% CI = (0.6781, 1.1742) / P value(Q) = 0.2641 / $I^2$ = 21.67%, 4) additive model (TT vs CC)→ RE OR=1.0478 / 95% CI = (0.5935, 1.8500) / P value(Q) = 0.0875 / $I^2$ = 45.6%.

Under the **co-dominant** model (**TC vs TT+CC**), there is **no heterogeneity** (variations are due to chance) since **P value(Q) = 0.25 / $I^2$ =22.55 %** and FE OR=0.776/ 95% CI = (0.625, 0.964) is **significant**, RE OR=0.771 / 95% CI = (0.600, 0.991) is also **significant** (fig. 1 Forest Plot). **Under-dominance**, which means that there is smaller chance for the heterozygotes to be affected by diabetes type 2, is established via **h-index**, h= ln (OR co-dominant)/|ln (OR additive)|=-5.43<-1.

We conduct a ***Sensitivity analysis***, in which the effect size is assessed by leaving out the study/ies with the biggest weight – w. After leaving out the heaviest study – (Mexico w = 15.44), **P value(Q) = 0.177 (no heterogeneity,** variations are due to chance**)**, FE OR=0.788/ 95% CI = (0.620, 1.003)→ marginally not significant, RE OR=0.788/ 95% CI = (0.576, 1.053) → not significant. After leaving out the 2 heaviest studies – (Mexico w = 15.44, India w = 15.17), **P value(Q) = 0.792 (no heterogeneity,** variations are due to chance**)**, FE OR=0.664/ 95% CI = (0.505, 0.874) → significant, RE OR=0.644/ 95% CI = (0.558, 0.744) → significant. After leaving out the 3 heaviest studies – (Mexico w = 15.44, India w = 15.17, Iraq w = 13.33), **P value(Q) = 0.669 (no heterogeneity,** variations are due to chance**)**, FE OR=0.674/ 95% CI = (0.490, 0.927) → significant, RE OR=0.654/ 95% CI = (0.528, 0.813) → significant. After leaving out the 4 heaviest studies – (Mexico w = 15.44, India w = 15.17, Iraq w = 13.33, Turkey w = 11.77), **P value(Q) = 0.581 (no heterogeneity,** variations are due to chance**)**, FE OR=0.723/ 95% CI = (0.492, 1.060) → not significant, RE OR=0.719/ 95% CI = (0.541, 0.956) → significant. Additionally, sensitivity analysis by MetaGenyo software showed that: a) Fixed Effect model OR = 0.78 / 95% CI = (0.62, 0,96)→ significant, b) Random Effect model OR = 0.77 / 95% CI = (0.60, 0,99) → significant, as seen in fig.2 and fig. 3.

The possibility of ***Publication bias*** was checked by Funnel Plot (fig. 4) and Egger's test, where the P value = 0.9578 > 0.05 which means that there is no significant publication bias.

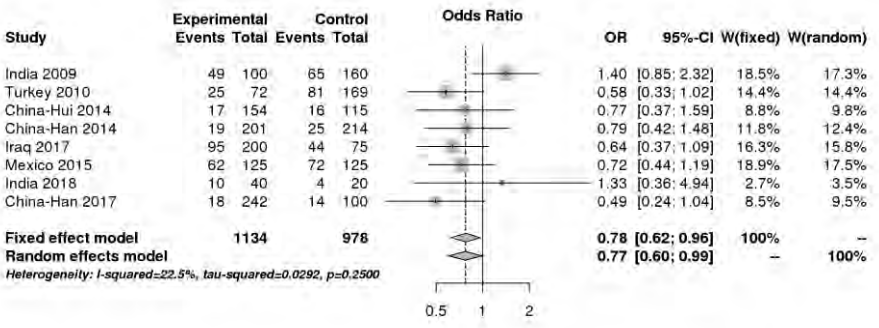


Fig. 1 Forest plot, Fixed Effect model and Random Effects model for TaqI polymorphism under the co-dominant model (TC vs TT+CC)

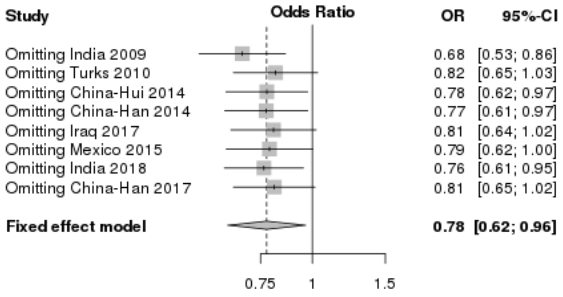


Fig. 2 Leave -1- out forest plot, Fixed Effect model under the co-dominant model

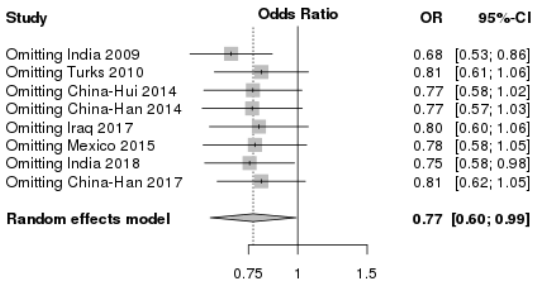


Fig. 3 Leave -1- out forest plot, Random Effect model under the co-dominant model

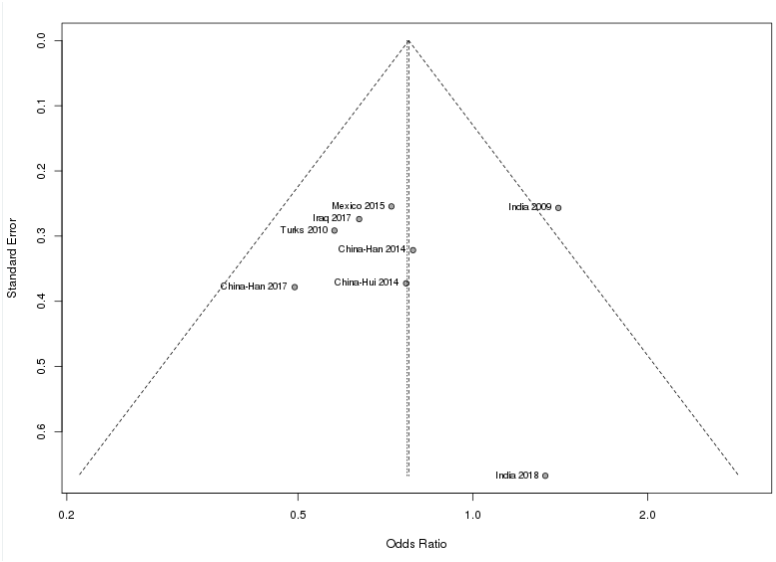


Fig. 4 Funnel Plot under the co-dominant model

Meta-analysis for BsmI polymorphism

| Studies | Distribution of **BsmI (rs 1544410)** VDR genotype | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| First author, ethnicity, year | Cases | | | Controls | | | HWE P-value | HWE adjusted P-value |
| | GG (BB) | GA (Bb) | AA (bb) | GG (BB) | GA (Bb) | AA (bb) | | |
| H. K. Bid, India (Caucasians), 2009 | 30 | 52 | 18 | 60 | 77 | 23 | 0.8309 | 0.8309 |
| P. N. Mukhopadhyaya, India (Caucasians), 2010 | 17 | 9 | 14 | 26 | 10 | 4 | 0.0733 | 0.2401 |
| R. J. Xu, China – Hui (descendants of Arabic & Persian merchants, over 12 million people in China), 2014 | 122 | 30 | 2 | 87 | 28 | 0 | 0.1372 | 0.2401 |
| R. J. Xu, China – Han (East Asians), 2014 | 176 | 24 | 1 | 172 | 47 | 0 | 0.0753 | 0.2401 |
| Errouagui, Maroc (Caucasians), 2014 | 18 | 57 | 60 | 18 | 57 | 61 | 0.4233 | 0.5926 |
| F. Yu, China – Han (East Asians), 2016 | 354 | 43 | 0 | 698 | 75 | 3 | 0.5201 | 0.6068 |
| D. Sarma, India (Caucasians), 2018 | 12 | 23 | 5 | 10 | 6 | 4 | 0.1276 | 0.2401 |
| Total | 729 | 238 | 100 | 1071 | 300 | 95 | | |
| Total number of cases & controls | 1067 | | | 1466 | | | | |

Table 2. Genotype distribution and Hardy-Weinberg Equilibrium for BsmI polymorphism

All the studies are in HWE (p≥0.05) as seen above (table 2). **If we consider Chinese Hui population as Caucasians** – with dark skin [including Indians and Moroccans], since they are descendants of Arabic & Persian merchants, the results of the meta-analysis under each genetic model show **no significant association.**

In detail: 1) allele contrast (A vs G), a] overall→ RE OR=1.0936 / 95% CI = (0.8137, 1.4697) / P value(Q) = 0.0132 / $I^2$ = 62.75%, b] Caucasians → RE OR=1.2595 / 95% CI = (0.8973, 1.7679) / P value(Q) = 0.0583 / $I^2$ = 56.13%, c] East Asians (Han Chinese) → RE OR=0.7915 / 95% CI = (0.4439, 1.4113) / P value(Q) = 0.0645 / $I^2$ = 70.73%, 2) recessive model (AA vs AG+GG), a] overall → RE OR= 1.1937 / 95% CI = (0.8387, 1.6990) / P value(Q) = 1.1957 / $I^2$ = 30.45%, b] Caucasians → FE OR=1.204 / 95% CI = (0.8419, 1.7218) / P value(Q) = 0.1202 / $I^2$ = 45.31%, c] East Asians (Han Chinese) → RE OR=0.8679 / 95% CI = (0.0984, 7.6560) / P value(Q) = 0.2678 / $I^2$ = 18.56%, 3) Dominant model (AA + AG vs GG), a] overall → RE OR=1.089 / 95% CI = (0.7590, 1.5629) / P value(Q) = 0.0269 / $I^2$ = 57.9%, b] Caucasians → FE OR=1.2509 / 95% CI = (0.9198, 1.7010) / P value(Q) = 0.1832 / $I^2$ = 35.7 %, c]

East Asians → RE OR=0.7684 / 95% CI = (0.3734, 1.5812) / P value(Q) = 0.0283 / $I^2$ =79.21 %, 4) Co-dominant (AG vs AA+GG), a] overall → RE OR=0.9597 / 95% CI = (0.7002, 1.3153) / P value(Q) = 0.0609 / $I^2$ = 50.2%, b] Caucasians → RE OR=1.0463 / 95% CI = (0.7924, 1.3816) / P value(Q) = 0.2732 / $I^2$ = 22.19 %, c] East Asians → RE OR=0.766 / 95% CI = (0.3407, 1.7220) / P value(Q) = 0.0148 / $I^2$ =83.18 %, 5) additive (AA vs GG), a] overall → FE OR=1.4762 / 95% CI = (0.9398, 2.3189) / P value(Q) = 0.3191 / $I^2$ = 14.52%, b] Caucasians → FE OR=1.515 / 95% CI = (0.9549, 2.4038) / P value(Q) = 0.2284 / $I^2$ = 28.97 %, c] East Asians → FE OR=0.829 / 95% CI = (0.0939, 7.3162) / P value(Q) = 0.2931 / $I^2$ = 9.53 %.

**If we consider Hui population as a genetically different ethnic group** that is distinct from Caucasians (their marriage practices tend towards endogamy leading to a distinct genetic pool), the results of the meta-analysis show that under the **dominant genetic model** there is a **statistically significant association in the subgroup of Caucasians [FE OR=1.4824 / 95% CI = (1.0309, 2.1317) / P value(Q) = 0.3528 / $I^2$ = 8.07 % (no heterogeneity]**.

The results in detail are: 1) allele contrast (A vs G), a] overall→ RE OR=1.0936 / 95% CI = (0.8137, 1.4697) / P value(Q) = 0.0132 / $I^2$ = 62.75%, b] Caucasians → RE OR=1.3803 / 95% CI = (0.9225, 2.0653) / P value(Q) = 0.0503 / $I^2$ = 61.54%, c] East Asians (Han Chinese) → RE OR=0.7915 / 95% CI = (0.4439, 1.4113) / P value(Q) = 0.0645 / $I^2$ = 70.73%, 2) recessive model (AA vs AG+GG), a] overall → RE OR= 1.1937 / 95% CI = (0.8387, 1.6990) / P value(Q) = 1.1957 / $I^2$ = 30.45%, b] Caucasians → RE OR=1.3137 / 95% CI = (0.6975, 2.4741) / P value(Q) = 0.0799 / $I^2$ = 55.64%, c] East Asians (Han Chinese) → FE OR=0.8679 / 95% CI = (0.0984, 7.6560) / P value(Q) = 0.2678 / $I^2$ = 18.56%, 3) **Dominant model (AA + AG vs GG)**, a] overall → RE OR=1.089 / 95% CI = (0.7590, 1.5629) / P value(Q) = 0.0269 / $I^2$ = 57.9%, b] **Caucasians → FE OR=1.4824 / 95% CI = (1.0309, 2.1317) → significant / P value(Q) = 0.3528 / $I^2$ = 8.07 % (no heterogeneity,** variations are due to chance**),** Egger's test (Caucasians) - P value = 0.3587 > 0.05 → not significant publication bias, c] East Asians → RE OR=0.7684 / 95% CI = (0.3734, 1.5812) / P value(Q) = 0.0283 / $I^2$ =79.21 % (fig. 5 Forest Plot), 4) Co-dominant (AG vs AA+GG), a] overall → RE OR=0.9597 / 95% CI = (0.7002, 1.3153) / P value(Q) = 0.0609 / $I^2$ = 50.2%, b] Caucasians → FE OR=1.153 / 95% CI = (0.8404, 1.5818) / P value(Q) = 0.315 / $I^2$ = 15.36 %, c] East Asians → RE OR=0.766 / 95% CI = (0.3407, 1.7220) / P value(Q) = 0.0148 / $I^2$ =83.18 %, 5) additive (AA vs GG), a] overall → FE OR=1.4762 / 95% CI = (0.9398, 2.3189) / P value(Q) = 0.3191 / $I^2$ = 14.52%, b] Caucasians → FE OR=1.4848 / 95% CI = (0.9308, 2.3686) / P value(Q) = 0.1498 / $I^2$ = 43.62 %, c] East Asians → FE OR=0.829 / 95% CI = (0.0939, 7.3162) / P value(Q) = 0.2931 / $I^2$ = 9.53 %.

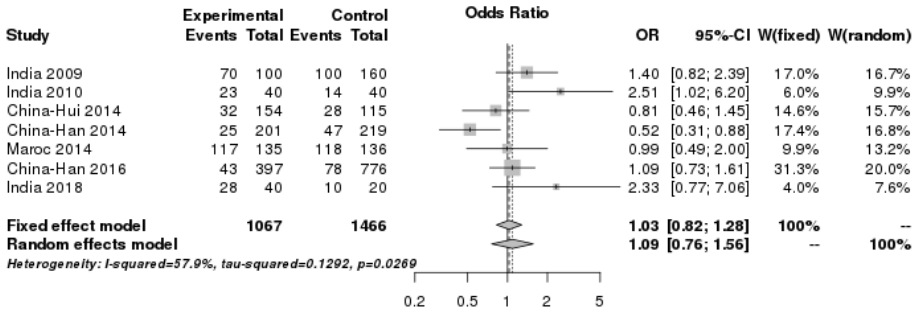


Fig. 5 Forest plot, Fixed Effect model and Random Effects model for BsmI polymorphism under the dominant model (AA+ AG vs GG)

**If we exclude the study (India 2010) – because it included 30 (out of 40) patients that are related** – in the subgroup analysis: in the subgroup of Caucasians (Morocco, India, Hui Chinese) → FE OR=1.142 / 95% CI = (0.823, 1.583) / RE OR=1.149 / 95% CI = (0.796, 1.659) → **not significant**, P value(Q) = 0.305 (**no heterogeneity**) and if Hui Chinese are not included in the subgroup of Caucasians (Morocco, India) → FE OR=1.339 / 95% CI = (0.900, 1.991) / RE OR=1.334 / 95% CI = (0.942, 1.891) → **not significant**, P value(Q) = 0.428 (**no heterogeneity**).

The Sensitivity analysis, in which the effect size is assessed by leaving out the study/ies with the biggest weight – w. After leaving out the heaviest study – (China-Han 2016 w = 24.79), **P value(Q) = 0.015 (heterogeneity)**, RE OR=1.116/ 95% CI = (0.700, 1.781) → not significant. After leaving out the 2 heaviest studies – (China-Han 2016 w = 24.79, China-Han 2014 w = 13.74), **P value(Q) = 0.183 (no heterogeneity,** variations are due to chance**)**, FE OR=1.251/ 95% CI = (0.920, 1.701) → not significant, RE OR=1.302/ 95% CI = (0.873, 1.940) →not significant. After leaving out the 3 heaviest studies – (China-Han 2016 w = 24.79, China-Han 2014 w = 13.74, India 2009 w = 13.46), **P value(Q) = 0.113 (no heterogeneity,** variations are due to chance**)**, FE OR=1.183/ 95% CI = (0.812, 1.723) → not significant, RE OR=1.316/ 95% CI = (0.754, 2.294) → not significant. After leaving out the 4 heaviest studies – (China-Han 2016 w = 24.79, China-Han 2014 w = 13.74, India 2009 w = 13.46, China-Hui 2014 w = 11.54), **P value(Q) = 0.204 (no heterogeneity,** variations are due to chance**)**, FE OR=1.557/ 95% CI = (0.949, 2.556) → not significant, RE OR=1.651/ 95% CI = (0.867, 3.144) → not significant. Additionally, the Sensitivity Analysis by MetaGenyo software result can be seen in fig. 6 and fig. 7.

The possibility of ***Publication bias*** was checked by Funnel Plot (fig. 8) and Egger's test → P value = 0.2884 > 0.05 → not significant publication bias.

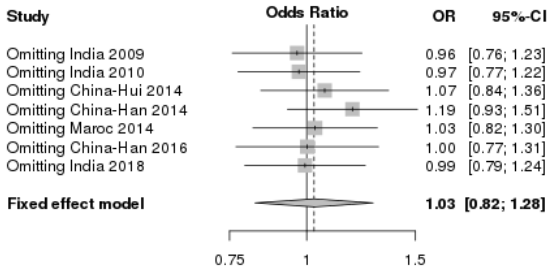


Fig. 6 Leave -1- out forest plot, Fixed Effect model under the dominant model

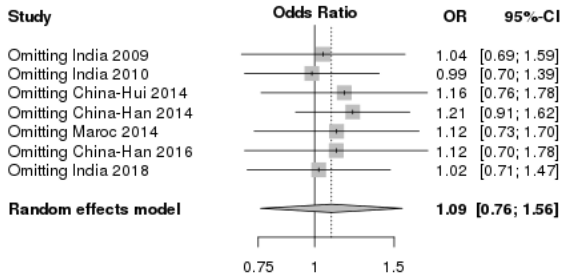


Fig. 7 Leave -1- out forest plot, Random Effect model under the dominant model

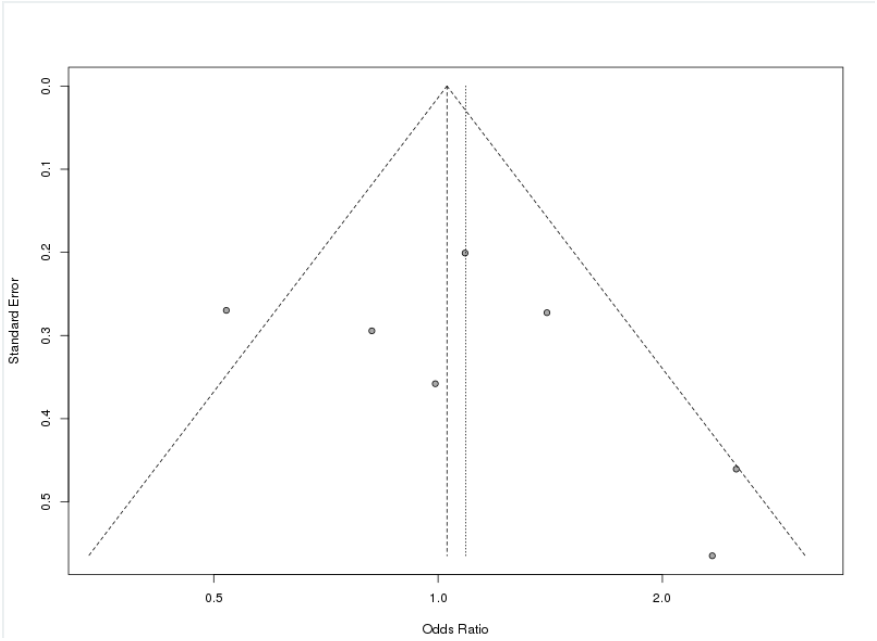


Fig. 8 Funnel Plot under the dominant model

Meta-analysis for FokI polymorphism

| Studies | Distribution of **FokI (rs 2228570)** VDR genotype | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| First author, ethnicity, year | Cases | | | Controls | | | HWE P-value | HWE adjusted P-value |
| | CC (FF) | CT (Ff) | TT (ff) | CC (FF) | CT (Ff) | TT (ff) | | |
| N.M. Al-Daghri, (Caucasians), 2012 | 22 | 133 | 213 | 19 | 111 | 129 | 0.4613 | 0.565 |
| Errouagui, Maroc (Caucasians), 2014 | 87 | 80 | 9 | 82 | 74 | 21 | 0.4962 | 0.565 |
| F. Yu, China – Han (East Asians), 2016 | 112 | 205 | 80 | 223 | 405 | 147 | 0.1236 | 0.3708 |
| I.Mahjoubi, Tunisia (Caucasians), 2016 | 231 | 180 | 28 | 168 | 117 | 17 | 0.565 | 0.565 |
| B.Angel, Chile(Mix), 2015 | 24 | 96 | 40 | 53 | 75 | 32 | 0.5601 | 0.565 |
| B.Angel, Chile(Mix), 2018 | 24 | 86 | 28 | 38 | 81 | 53 | 0.5042 | 0.565 |
| Z. Xia, China – Han (East Asians), 2017 | 129 | 94 | 19 | 38 | 50 | 12 | 0.4684 | 0.565 |
| Total | 637 | 874 | 449 | 623 | 916 | 426 | | |
| Total number of cases & controls | 1960 | | | 1965 | | | | |

Table 3. Genotype distribution and Hardy-Weinberg Equilibrium for FokI polymorphism

All the studies are in HWE (p≥0.05) as seen above (table 3). The results of the meta-analysis show that under the **co-dominant genetic model (CT vs CC+TT)** [FE OR=1.775/ 95% CI = (1.2916, 2.4393) / P value(Q) = 0.7842 / $I^2$ = 0%] **and under the model (CC vs CT)** [FE OR=0.4536/ 95% CI = (0.3007, 0.6845)/ P value(Q) = 0.2158 / $I^2$ = 34.74%] there is a **statistically significant association in the subgroup of Mixed population**.

The results of the meta-analysis under each ***genetic model*** in detail are: **1)** allele contrast (C vs T), a] overall→ RE OR=0.9805 / 95% CI = (0.8105, 1.1862) / P value(Q) = 0.0018 / $I^2$ = 71.43%, b] Caucasians → RE OR=0.9480 / 95% CI = (0.7373, 1.2190) / P value(Q) = 0.0726 / $I^2$ = 61.88%, c] East Asians (Han Chinese) → RE OR=1.1993 / 95% CI = (0.7483, 1.9219) / P value(Q) = 0.015 / $I^2$ = 83.11%, d] Mix→ RE OR=0.8397/ 95% CI = (0.4749, 1.4848) / P value(Q) = 0.0104 / $I^2$ = 84.75%, **2)** recessive model (CC vs CT+TT), a] overall → RE OR= 0.8948 / 95% CI = (0.6586, 1.2157) / P value(Q) = 0.0012 / $I^2$ = 72.68%, b] Caucasians → FE OR=0.9396 / 95% CI = (0.7503, 1.768) / P value(Q) = 0.5612 / $I^2$ = 0%, c] East Asians (Han Chinese) → RE OR=1.3049 / 95% CI = (0.6924, 2.4592) / P value(Q) = 0.0198 / $I^2$ = 81.57%, d] Mix→ RE OR=0.5118/ 95% CI = (0.2493, 1.0509) / P value(Q) = 0.0677 / $I^2$ = 70.05%, **3)** Dominant model (CC + CT vs TT), a] overall → RE OR=1.0772 / 95% CI = (0.7906, 1.4675) / P value(Q) = 0.0133 / $I^2$ = 62.69%, b] Caucasians → RE OR=0.0690 / 95% CI = (0.5602, 2.0400) / P value(Q) = 0.0203 / $I^2$ = 74.34 %, c] East Asians → FE OR=0.9994 / 95% CI = (0.7535, 1.3255) / P value(Q) = 0.1933 / $I^2$ =40.91 %, d] Mix→ RE OR=1.1458 / 95% CI = (0.4995,2.6280) / P value(Q) = 0.0258 / $I^2$ = 79.87%, **4) Co-dominant (CT vs CC+TT),** a] overall → RE OR=1.0787 / 95% CI = (0.8420, 1.3819) / P value(Q) = 0.0033 / $I^2$ = 69.33%, b]Caucasians → FE OR=0.9709 / 95% CI = (0.7987, 1.1803) / P value(Q) = 0.1614 / $I^2$ = 45.18 %, c] East Asians → FE OR=0.8914 / 95% CI = (0.7188, 1.1054) / P value(Q) = 0.1114 / $I^2$ =60.53 %, d] **Mix→ FE OR=1.775/ 95% CI = (1.2916, 2.4393) / P value(Q) = 0.7842 / $I^2$ = 0% → *significant***, 4) additive (CC vs TT), a] overall → RE OR=0.9872 / 95% CI = (0.6504, 1.4985) / P value(Q) = 0.0055 / $I^2$ = 67.24%, b] Caucasians → RE OR=1.0797 / 95% CI = (0.5363, 2.1737) / P value(Q) = 0.0509 / $I^2$ = 66.41 %, c] East Asians → RE OR=1.2966 / 95% CI = (0.5765, 2.9162) / P value(Q) = 0.0612 / $I^2$ = 71.47 %, d] Mix→ RE OR=0.6565/ 95% CI = (0.2038, 2.1153) / P value(Q) = 0.0147 / $I^2$ = 83.21%, **5) (CC vs CT),** a] overall → RE OR=0.8713 / 95% CI = (0.6399, 1.1864) / P value(Q) = 0.0024 / $I^2$ = 70.49%, b] Caucasians → FE OR= 0.9272 / 95% CI = (0.7334, 1.1722) / P value(Q) = 0.9346 / $I^2$ = 0 %, c] East Asians → RE OR=1.2903 / 95% CI = (0.7208, 2.3099) / P value(Q) = 0.0406 / $I^2$ = 76.16 %, d] **Mix→ FE OR=0.4536/ 95% CI = (0.3007, 0.6845) / P value(Q) = 0.2158 / $I^2$ = 34.74% → *significant***.

**Over-dominance** (bigger chance for the heterozygotes to be affected by diabetes type 2) in the mixed population (cases-298, controls-332) is established via **h-index**, h= ln (OR co-dominant)/|ln (OR additive)|=+1.3675>+1.

We conduct a Sensitivity analysis (the effect size is assessed by leaving out the study/ies with the biggest weight – w). After leaving out the heaviest study – (China-Han 2016 w = 65.54), **P value(Q) = 0.002 (heterogeneity)**, RE OR=1.106/ 95% CI = (0.808, 1.514) → not significant. After leaving out the 2 heaviest studies – (China-Han 2016 w = 65.54, Tunisia 2016 w = 42.79), **P value(Q) = 0.001 (heterogeneity)**, RE OR=1.111/ 95% CI = (0.738, 1.672) → not significant. After leaving out the 3 heaviest studies – (China-Han 2016 w = 65.54, Tunisia 2016 w = 42.79, Saudi 2012 w = 36.31), **P value(Q) = 0.005 (heterogeneity,** variations are due to chance**)**, RE OR=1.239/ 95% CI = (0.780, 1.967) → not significant. The Sensitivity Analysis by MetaGenyo software is shown in fig.9 and fig.10.

The possibility of ***Publication bias*** was checked by Funnel Plot (fig. 11) and Egger's test P value = 0.6933 → not significant publication bias.

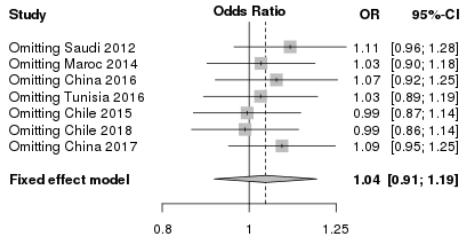


Fig. 9 Leave -1- out forest plot, Fixed Effect model under the overdominant model

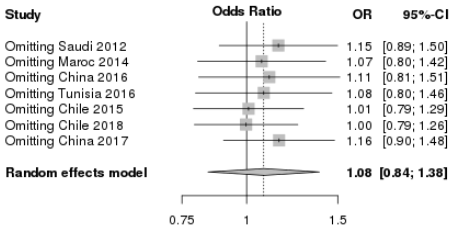


Fig. 10 Leave -1- out forest plot, Fixed Effect model under the overdominant model

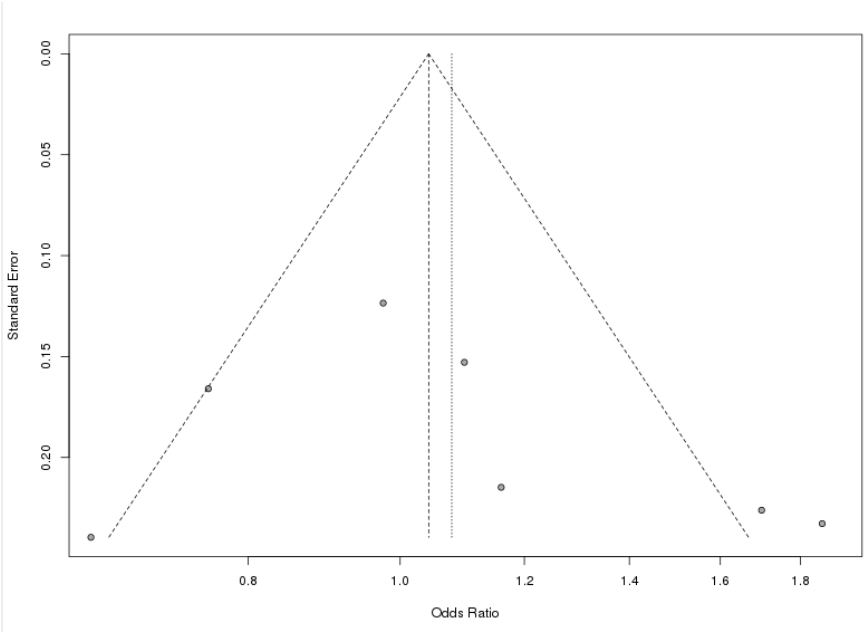


Fig. 11 Funnel Plot under the overdominant model

Meta-analysis for ApaI polymorphism

| Studies | Distribution of **ApaI (rs 7975232)** VDR genotype | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| First author, ethnicity, year | Cases | | | Controls | | | HWE P-value | HWE adjusted P-value |
| | TT (AA) | TG (Aa) | GG (aa) | TT (AA) | TG (Aa) | GG (aa) | | |
| F. Dilmec, Turkey (Caucasians), 2010 | 27 | 38 | 7 | 61 | 82 | 26 | 0.8566 | 0.8566 |
| Errouagui, Maroc (Caucasians), 2014 | 36 | 89 | 34 | 36 | 90 | 34 | 0.1133 | 0.307 |
| Z. Xia, China – Han (East Asians), 2017 | 19 | 92 | 131 | 13 | 38 | 49 | 0.2047 | 0.307 |
| Total | 82 | 219 | 172 | 110 | 210 | 109 | | |
| Total number of cases & controls | | | 473 | | | 429 | | |

Table 4. Genotype distribution and Hardy-Weinberg Equilibrium for ApaI polymorphism

All the studies are in HWE (p≥0.05) as seen above (table 4). No significant association was detected.

The results of the meta-analysis under each ***genetic model***, are: 1) allele contrast (G vs T)→ FE OR=1.0422 / 95% CI = (0.8508, 1.2767) / P value(Q) = 0.3315 / $I^2$ = 9.43% and in Caucasians → FE OR=0.9456 / 95% CI = (0.7393, 1.2095) / P value(Q) = 0.5636 / $I^2$ = 0%, 2) recessive model (GG vs GT+TT)→ FE OR=1.0330 / 95% CI = (0.7449, 1.4325) / P value(Q) = 0.3577 / $I^2$ = 2.73% and in Caucasians → FE OR=0.8739 / 95% CI = (0.5526, 1.3820) / P value(Q) = 0.3136 / $I^2$ = 1.5%, 3) dominant model (GG+GT vs TT)→ FE OR=1.0976 / 95% CI = (0.7787, 1.5473) / P value(Q) = 0.3811 / $I^2$ = 0% and in Caucasians → FE OR=0.9684 / 95% CI = (0.6580, 1.4254) / P value(Q) = 0.8948 / $I^2$ = 0%, 4) additive model (GG vs TT)→ RE OR=1.0926 / 95% CI = (0.6998, 1.7060) / P value(Q) = 0.2004 / $I^2$ = 37.79% and in Caucasians → FE OR=0.8497 / 95% CI = (0.4934, 1.4633) / P value(Q) = 0.4001 / $I^2$ = 0%, 5) co-dominant (GT vs TT+GG) → FE OR=1.0404/ 95% CI = (0.7861, 1.3770) /P value(Q) = 0.8644 / $I^2$ =0 % and in Caucasians → FE OR=1.0616 / 95% CI = (0.7517, 1.4993) / P value(Q) = 0.615 / $I^2$ = 0%.

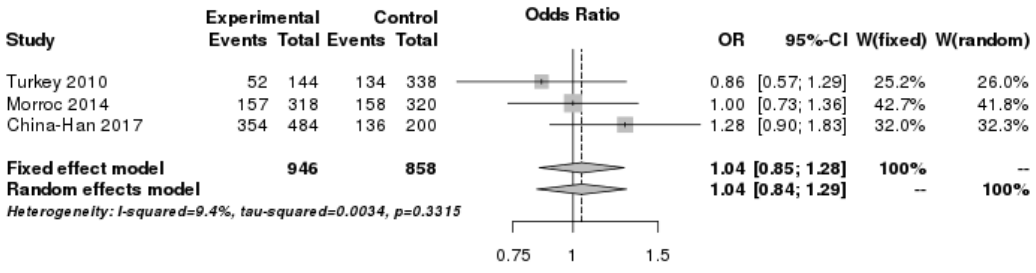


Fig. 12 Forest plot, Fixed Effect model and Random Effects model for BsmI polymorphism under allele contrast (G vs T)

Sensitivity Analysis by MetaGenyo software in fig. 13 and fig.14. The possibility of ***Publication bias*** was checked by Funnel Plot (fig. 11) and Egger's test → P value = 0.8532 → not significant publication bias.

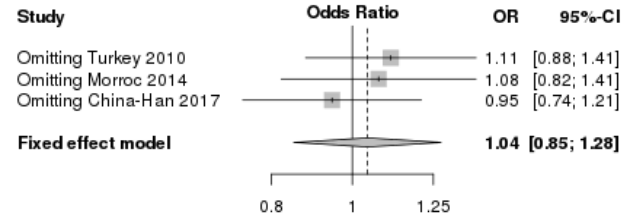


Fig. 13 Leave -1- out forest plot, Fixed Effect model under the allele contrast

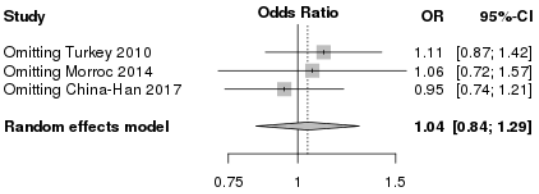


Fig. 14 Leave -1- out forest plot, Random Effect model under the allele contrast

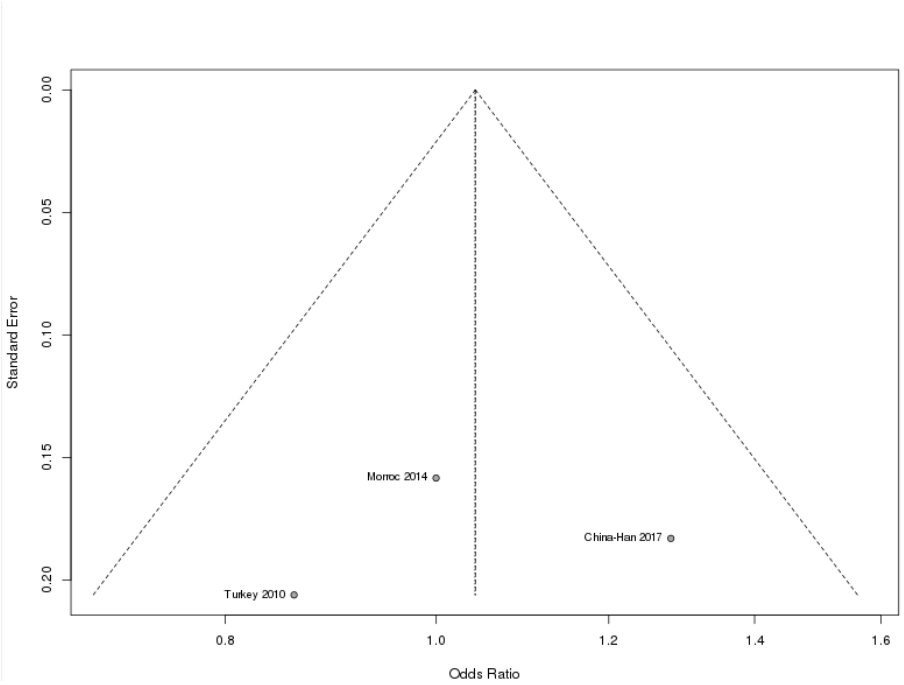


Fig. 15 Funnel Plot under allele contrast (G vs T)

CONCLUSIONS

According to Zintzaras E. et al. in 2008 ([26]), meta-analysis provides a robust tool to investigate contradictory results in genetic association studies by estimating population-wide effects of genetic risk factors in diseases and explaining sources of bias and heterogeneity.

For **TaqI polymorphism**, overall (Caucasians – with dark skin & East Asians) the results indicate that heterozygotes (**TC**) are protected of type 2 diabetes, since there is 22,4% smaller chance for the heterozygote to be affected by the disease than for the homozygotes (**TT+CC**) [FE OR=0.776/ 95% CI = (0.625, 0.964) is **significant**, RE OR=0.771 / 95% CI = (0.600, 0.991) is also **significant**]. In favor of these results is the fact that 1134 cases & 978 controls were included, there was **no heterogeneity** (**P value(Q) = 0.25 / $I^2$ =22.55 %**) and no significant publication bias was detected (as indicated by Egger's test: P value = 0.9578 >

0.05 and a funnel plot). On the other hand, the sensitivity analysis shows contradicting results, although Fixed Effect model OR = 0.78 / 95% CI = (0.62, 0,96)→ significant and Random Effect model OR = 0.77 / 95% CI = (0.60, 0,99) → significant.

TaqI polymorphism is located inside exon 9 and the protein coded remains the same. It has been shown by C. Andraos et al. in 2011 [29] that the variant is inside CpG5 of CGI1060 and the C allele is always methylated gradually reducing vdr protein levels and additionally it has been stated by D. Saccone et al. in 2015 [30] that C allele(t) is associated with lower levels of vdr protein and TT genotype is associated with higher levels of vdr protein ([31]). However, it is known ([20]) that low levels of vdr protein is associated with dysfunction of β cells and type 2 diabetes. The above can explain why CC genotype has higher risk of type 2 diabetes than TC. On the other hand, there has been an indication that T allele is associated with obesity in Greek population ([32]) so it is possible that TT genotype is increasing the risk of diabetes via obesity, since vdr protein is an important transcription factor and obesity-induced β cell dysfunction is poorly understood.

For **BsmI polymorphism,** in Caucasians – with dark skin [Hui not included] (subgroup analysis) the results indicate that carriers of the A allele (AA+AG) have 48,2% higher risk of acquiring type 2 diabetes [FE OR=1.4824 / 95% CI = (1.0309, 2.1317)] which is significant. In favor of these results is the fact that there was no heterogeneity**,** which means that variations are due to chance (because P value(Q) = 0.3528 / $I^2$ = 8.07 %) and no significant publication bias was detected (as indicated by Egger's test: P value = 0.3587 > 0.05 and a funnel plot). The results should be viewed with caution mainly because no significant association has been shown after the exclusion of the study that included relatives and additionally because of the small number of participants (315 cases & 356 controls).

For **FokI polymorphism**, in Mixed population – (**subgroup analysis**) the results indicate that heterozygotes CT have 77,5% higher risk of acquiring type 2 diabetes compared to the homozygotes (CC+TT) [FE OR=1.775/ 95% CI = (1.2916, 2.4393)] which is significant. In favor of these results is the fact that there was **no heterogeneity,** which means that variations are due to chance in CT vs CC+TT model **(P value(Q) = 0.7842 / $I^2$ = 0%).** Additionally, heterozygotes CT have 102,5% higher risk of acquiring type 2 diabetes compared to the homozygotes CC [ CC vs CT, FE OR=0.4536/ 95% CI = (0.3007, 0.6845)] which is significant but there was **medium heterogeneity** (**P value(Q) = 0.2158 / $I^2$ = 34.74%).** On the other hand, the number of participants (298 cases & 332 controls) is small.

FokI polymorphism is located inside the exon 2 and the C allele codes a shorter protein that has a higher trans-activational capacity – as shown be Arai et al. in 1997 [33] – and therefor FokI may indirectly affect VDR regulation through autoregulation. As stated by D. Saccone et al. in 2015 [30], the risk presumably is modified by influencing VDR protein levels and VDR trans-activation capacity.

For **ApaI polymorphism** no significant associations were found. There was no heterogeneity under each genetic model [except for the additive model (GG vs TT) where medium heterogeneity was found ($I^2$ = 37.79%)]. No significant publication bias was detected (as indicated by Egger's test: P value = 0.9578 > 0.05 and a funnel plot). On the other hand, since only 3 studies were included, the number of participants (473 cases & 429 controls) is small and ApaI polymorphism is located inside intron 8 (between exon 8 & 9) and no known functional consequence has been described.

Consequently, further analysis should be conducted to shed light on the exact association of VDR with type 2 diabetes. This meta-analysis though, does indicate a possible association.

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